

Application No.: 10/509,144
Attorney Docket No.: P098US
First Applicant's Name: Kurt Berlin
Application Filing Date: 27 September 2004
Date of Office Action: 12 November 2009
Date of Response: July 16, 2010
Examiner: Steven C. Pohnert

IN THE CLAIMS:

1. (Previously presented) A method for cytosine methylation analysis of genomic DNA wherein the genomic template DNA is amplified such that the cytosine methylation pattern of the genomic template DNA is retained in the amplificate sequence(s), said method comprising the following steps:

(a) providing a sample of DNA, said DNA being methylated at one or more cytosine positions;

(b) heating the genomic DNA to a temperature operative to cause denaturation;

(c) cooling the denatured DNA in the presence of single stranded oligonucleotide primers such that the primers anneal to the DNA;

(d) heating the mixture in the presence of a polymerase and nucleotides to a temperature such that the primers are extended, thereby resulting in hemimethylated DNA;

(e) contacting the hemimethylated DNA with a methyltransferase and a methyl donor molecule under conditions conducive to the methylation of the synthesised strand such that the CpG dinucleotides within the synthesised strand are methylated according to the methylation status of the corresponding CpG dinucleotide on the template strand thereby preserving the genomic methylation pattern;

(f) repeating steps (b)-(e) a plurality of times to reach a plurality of nucleic acids, whereby each of said nucleic acids is methylated at the same one or more cytosine positions as the DNA provided in step (a); and

(g) analyzing the methylation of the nucleic acids of step (f) whereby the methylation of the DNA of the sample of step (a) is deduced.

2. (Previously presented) A method according to Claim 1 wherein the methyltransferase is a maintenance methyltransferase.

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3. (Previously presented) A method according to Claim 1 wherein the methyltransferase is DNA (cytosine-5) Methyltransferase (DNMT 1).

4. (Previously presented) A method according to Claim 1 wherein the methyl donor molecule is S-adenosylmethionine.

5. (Previously presented) A method according to Claim 1 wherein the methylated CpG dinucleotides carry a detectable label which is incorporated into the synthesised nucleic acid strand.

6. (Previously presented) A method according to Claim 1 wherein a plurality of primer oligonucleotides are immobilised on a solid surface.

7. (Previously presented) A method according to Claim 1 wherein the methyltransferase is immobilised on a solid surface.

8. (Previously presented) A method according to Claim 1 wherein the polymerase is immobilised on a solid surface.

9. (Previously presented) A method according to Claim 1 further comprising Step (g) a treatment with an agent capable of distinguishing between methylated and unmethylated cytosine bases.

10. (Previously presented) A method according to Claim 9 wherein the agent is a methylation sensitive restriction enzyme.

11. (Previously presented) A method according to Claim 9 wherein the agent is a bisulphite solution.

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12. (Previously withdrawn) A device for the methylation pattern retaining amplification of nucleic acids according to Claim 1 said device comprising two or more reaction chambers, channel means providing fluid connections between adjacent chambers and the first and last reaction chambers, temperature regulating means for controlling the temperature of each reaction chamber.

13. (Previously withdrawn) A device for the methylation pattern retaining amplification of nucleic acids according to Claim 1 to 6 comprising

two vessels,
a reaction chamber,
temperature regulating means for controlling the temperature of the reaction chamber,
means for transferring liquid reagent from the first and second vessels to the reaction chamber,

channel means providing fluid connections between adjacent chambers and the first and last reaction chambers; and

means for draining liquid reagents from the reaction chamber.

14. (Previously withdrawn) A nucleic acid obtainable by a method according to one of the claims 1 to 11.

15. (Previously withdrawn) A method of manufacturing a methylated nucleic acid using a method according to one of the claims 1 to 11.